IN THE SPECIFICATION

Please substitute the following paragraphs as indicated:

At page 7, full paragraph 7:

Fig. 11 shows the stability of PLAS*min*™ DNA complexes upon freezing and lyophilization. Particles were tested with sucrose, trehalose, or no excipient. Particles were tested with and without polyethylene glycol, and with TFA or acetate as the counterion. DNA stability was assessed by a low (3400 x g x 1 min) spin to pellet aggregates, and monitoring the absorbance of DNA in the supernatant. Stability with acetate as the counterion surpassed other formulations in the absence of excipient.

At page 8, full paragraph 2:

Fig. 15 shows the results of gene transfer experiments using lyophilized PLAS*min*™ DNA complexes. Luciferase enzyme was encoded by the complexes and its activity was measured as a means of monitoring gene transfer. While sucrose and trehalose were effective in protecting the gene transfer activity to all particles, particles which contained polyethylene glycol (10 kdal) and acetate as a counterion were surprisingly stable to lyophilization, even in the absence of cryoprotectant excipient (disaccharide).

At page 15, full paragraph 3:

Resistance to serum nucleases is, among other properties, an important feature of any effective gene therapy vector designed to be administered systemically. Ideally, engineering this resistance should not compromise other desirable properties of a vector, such as its small size and colloidal stability. We have developed reagents and methods that permit us to reproducibly compact plasmid DNA with polylysine-polyethylene glycol (PEG) conjugates to form small particles having defined morphology (PLAS*min*™ DNA complexes). Some of these formulations are stable in serum and do not aggregate in physiologic saline. By changing components and conditions of the compaction procedure, size and shape of the particles can be modified. To evaluate potential correlations between serum stability and the physical state of PLAS*min*™ DNA complexes, we have prepared a matrix of 24 formulations using polylysines of various lengths and substituted with PEG to various extents. Fig. 9D. Polylysines having exactly 15, 30, and 45 residues were obtained by solid-phase synthesis. These polymers contained

an N-terminal cysteine residue that was used to conjugate PEG. Various mixtures of PEG-substituted and non-substituted polylysines we re used to obtain different PLAS*min*^{TM} DNA complexes. Stability of the complexes in 75% mouse serum was tested by incubating compacted DNA at 37 ^{o}C for up to 5 days and determining half-life of DNA degradation. Simultaneously, physical characteristics of the complexes in 150 mM NaCl were determined. Morphology was visualized by transmission electron microscopy (Fig. 10 and Fig. 17). DNA condensed with acetate and bicarbonate salts of CK30 polylysine assumed forms of long (100-300 nm) and narrow (10-20 nm) rods and relaxed toroids (~50-100 nm diameter, 10-20 nm width); the TFA salt resulted in much shorter rods (<60 nm by 20-30 nm) and small globules (20-30 nm); the chloride form of CK30 did not compact DNA at all (Fig. 10), while CK45/chloride (Fig. 17) gave results similar to CK30/acetate. Colloidal instability (tendency to aggregate) was evaluated by a sedimentation assay. Additionally, light scattering of solutions containing PLAS*min*^{TM} DNA complexes was measured and expressed as a turbidity parameter (Fig. 8). We found that all PLAS*min*^{TM} DNA complexes (Fig. 9A) were much more stable in serum than naked DNA. The half-life for compacted DNA ranged from ~2-17 hr, while naked DNA was completely digested within a few minutes. We also found a correlation (r^2=0.77) between half-life of degradation and colloidal instability of PLAS*min*^{TM} DNA complexes: particles that tended to aggregate were more resistant to nucleases. The tendency to aggregate also correlated with morphology of the complexes: rod-like complexes did not aggregate; thus, they all showed very similar serum stability, independent of their composition ($t_{1/2}$~2-5 hr). In contrast, spherical complexes showed various extents of tendency to aggregate depending on polylysine chain-length and PEG content. There was little difference in serum stability between small globules and rod-like particles. In agreement with the prediction that aggregated particles should scatter various light wavelengths differently than small complexes, we found a good correlation (r^2=0.88) between colloidal instability of PLAS*min*^{TM} DNA complexes and turbidity of their solutions (Fig. 9B): stable complexes had turbidity parameter around –4 to –5 (in accordance with the Rayleigh law), while for the largest and least stable particles this value increased to –1.3. Consequently, the turbidity parameter also correlated with the half-life of DNA degradation in serum (r^2=0.73; Fig. 9C). Thus, we conclude that the turbidity parameter, which is easy to determine, can be conveniently used to preliminarily screen various formulations of compacted DNA and predict their colloidal stability as well as serum stability.

At page 17, full paragraph 1:

Effective gene transfer to lung would facilitate therapies for pulmonary diseases, such as cystic fibrosis, and may provide a potent means for administering mucosal vaccines. Although direct instillation of naked DNA into mouse airways generates measurable transgene expression, the level of expression is low, and the duration of expression is short. We have developed reagents and formulation methods that compact single molecules of plasmid DNA into 20-25 nm particles (PLAS*min*^{TM} DNA complexes). Unlike naked DNA, these complexes are protected from nuclease digestion and are stable in serum. Additionally, PLAS*min*^{TM} DNA complexes do not aggregate in physiologic saline and can be concentrated to over 12 mg/ml of DNA. To determine if PLAS*min*^{TM} DNA complexes would generate significant levels of gene expression in lung, we instilled naked and PLAS*min*^{TM} DNA complexes into the lungs of C57BL/6J mice via direct intratracheal administration. These compacted particles consisted of plasmid DNA and PEG-substituted polylysine polymers consisting of 30 lysine residues. The plasmid construct encoded a luciferase reporter gene transcriptionally controlled by a CMV enhancer, an elongation factor 1-alpha (EF1-alpha) promoter, EF1-alpha intron 1, the RU5 translational enhancer from HTLV I, and an SV40 late polyadenylation signal. A DNA dose of 100 ug was administered in 25 or 50 ul of 150 mM NaCl. At 2, 4, 5, or 12 days following gene transfer, extracts were prepared from both lungs and luciferase activity was measured as relative light units per mg of protein (Fig. 6). Whereas naked DNA generated a signal of approximately 4,000 RLU/mg on day 2 and 1,100 RLU/mg on day 4, PLAS*min*^{TM} DNA complexes generated approximately 1,100,000 RLU/mg on day 2, and 630,000 rlu/mg on day 4. Gene expression persisted for at least 12 days after gene transfer, although at lower levels. These compacted DNA particles produced 400-fold enhanced gene expression compared to naked DNA on day 2, and over 1,300-fold improved gene expression on day 4. In contrast to whole lung extracts, less gene expression was noted in trachea, and no expression in liver (data not shown). In dose response studies, peak levels of transgene expression was observed using a 100 ug dose (Fig. 7). In summary, we have determined that PLAS*min*^{TM} DNA complexes effectively deliver and express transgenes in mouse lung following direct intra-tracheal administration. In studies in progress, the beta-galactosidase reporter gene is being utilized to define the cell type(s) being transfected. PLAS*min*^{TM} DNA complexes may provide an appropriate gene transfer method for diverse pulmonary diseases and/or mucosal vaccines.

At page 18, full paragraph2 :

To optimize formulations of PLAS*min*^{TM} DNA complexes for intramuscular administration, various preparation of compacted DNA encoding the luciferase reporter gene were administered to CD2 mice by single injection in the tibialis anterior muscle. Gene expression was assayed at various days post gene transfer and is presented as relative light units (RLU)/mg protein. In Figure 1, expression of compacted DNA formulated with the acetate salt of CK30 polycation (complexed with PEG 10 kD) was enhanced, as measured by luciferase activity on both days 1 and 3, compared to other preparations of DNA formulated with the TFA salt of CK30 or CK45. To define further the roles of counterion type, length of polylysine, and percent substitution of polyethylene glycol (PEG), additional experiments were conducted. Animals received IM injections of TFA complexes consisting of either CK30 or CK45, and PEG sizes of either 5 or 10 kD. Figure 2) Luciferase activity was significantly less than that observed for CK30, PEG 10 kD, acetate complexes in Figure 1. The enhanced gene expression of complexes prepared using the acetate salt of CK30, PEG 10 kD, was confirmed. (Figure 3) In this experiment, the CK30 polycation generated better luciferase activity than the CK45 polymer, and CK30 yielded higher levels of luciferase activity when complexed with 10 kD rather than 5 kD PEG. The duration of gene expression produced by acetate complexes consisting of either CK30 or CK45, both complexes with PEG 10 kD, were next evaluated, and the results are shown in Figure 4. In this study, the CK30 polycation gave the best level of reporter gene activity, and the level of activity was better on day 7 than days 1 or 3. A variety of acetate complexes were tested for gene activity as shown in Figure 5. These formulations included CK15, CK30, and CK45 polycations complexed with various percentages of PEG 10 kD. A time course to 30 days was performed. Although gene expression on days 1, 3, and 7 appeared better using CK15 compared to CK30, the particle sizes of some CK15 complexes were larger than 30 nm or two times the theoretical diameter of a complex of said single nucleic acid molecule and a sufficient number of polycation molecules to provide a charge ratio of about 1:1, in the form of a condensed sphere. For days 1, 3, 7, and 15, at least one preparation of CK30 compacted DNA was superior to any CK45 preparation. For CK30, the 100% PEG 10 kD complexes generated better reporter gene activity than either the 70% or 40% substitutions. In summary, the best formulation of compacted DNA in these studies was the acetate salt of CK30 polycation having a 100% substitution with PEG 10 kD.

At page 20, full paragraph 1:

The stability of PLAS*min* ™ DNA complexes upon freezing and lyophilization was assessed. Particles were tested with sucrose, trehalose, or no excipient. Particles were tested with and without polyethylene glycol, and with TFA or acetate as the counterion to the polyethylene glycol. DNA stability was assessed by a low (3400 x g x 1 min) spin to pellet aggregates, and monitoring the absorbance of DNA in the supernatant. See Fig. 11. Stability of the complexes with acetate as the counterion surpassed other formulations in the absence of excipient.

At page 21, full paragraph 2:

Gene transfer experiments using lyophilized and rehydrated PLAS*min*™ DNA complexes were performed, comparing them to pre-lyophilization preparations. Luciferase enzyme was encoded by the complexes and its activity was measured as a means of monitoring gene transfer. While sucrose and trehalose were effective in protecting the gene transfer activity to all particles, particles which contained polyethylene glycol (10 kdal) and acetate as a counterion were surprisingly stable to lyophilization, even in the absence of cryoprotectant excipient (disaccharide). See Fig. 15.